

“Development Of CMC Hydrogel Loaded with Tridax Procumbens Leaf Extract and It’s Application”

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Abstract

The present study focuses on the formulation and application of a CMC-based hydrogel enriched with Tridax procumbens leaf extract for wound healing and fruit-coating purposes. Carboxymethyl cellulose (CMC), known for its biocompatibility, moisture retention ability, and gel-forming properties, was cross-linked with citric acid to develop a stable hydrogel system. Tridax procumbens, a medicinal herb with proven antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties, was incorporated into the hydrogel to enhance its therapeutic potential.

Phytochemical screening of the leaf extract confirmed the presence of flavonoids, tannins, saponins, and terpenoids, which contribute to antimicrobial and regenerative activities. The hydrogel was prepared, homogenized, and incubated to allow proper infusion of plant bioactive compounds. The formulated gel was tested for antibacterial activity, showing effective inhibition against selected microbial strains. Additionally, the hydrogel was evaluated for fruit-coating efficiency, demonstrating its ability to prevent spoilage and maintain freshness.

Overall, the study concludes that CMC hydrogel enriched with Tridax procumbens extract is a promising natural biomaterial with significant potential in wound healing applications and biodegradable fruit preservation. The combination of CMC and medicinal plant extract provides a safe, eco-friendly, and effective alternative to synthetic gels and chemical preservatives.

AIMS AND OBJECTIVE

- ❖ Collection of *Tridax Procumbens* leaves.
- ❖ Extraction of *Tridax Procumbens* Plants.
- ❖ Purification of the *Tridax procumbens* Plants.
- ❖ Screening of antimicrobial activity against selected pathogens.
- ❖ Developments of CMC hydrogel containing *Tridax Procumbens* Herbal formulation.
- ❖ Phytochemical Screening.
- ❖ Fruit coating with CMC hydrogel.

INTRODUCTION

Tridax procumbens, a plant belonging to the daisy family, is found perennially in various tropical and subtropical regions as well as mildly temperate regions worldwide (Mundada and Shivhare, et al., 2010).

Scientific studies have reported that extracts of *Tridax procumbens* possess a wide range of pharmacological Activities including mosquito repellent, wound healing, anti-inflammatory, hepatoprotective, leishmanicidal, antioxidant, immunomodulatory, and anti-protozoal effects (Suseela et al., 2002).

The plant produces several secondary metabolites which contribute to its medicinal value, making it a potential source for natural remedies and pharmaceutical applications. Cellulose, one of the most abundant natural polymers found in cotton, wood, and grass, is widely utilized due to its renewability and ability to be chemically modified. Carboxymethyl cellulose (CMC), a water-soluble derivative of cellulose, is extensively used in food, pharmaceutical, textile, and biomedical industries.

CMC is known for its excellent hydrophilic and gelling properties, and when cross-linked with citric acid and other materials, it forms hydrogel structures that have potential applications in drug delivery and wound care.

In India, herbal remedies have been used for centuries for the treatment of skin diseases and wound healing. Natural hydrogels formed from polysaccharides like cellulose offer a moist environment that promotes healing, disinfection, and tissue regeneration.

Thus, combining *Tridax procumbens* with biodegradable cellulose-based hydrogels presents a promising approach for developing efficient, eco-friendly therapeutic formulations.

In the present study, we aim to investigate the medicinal potential of *Tridax procumbens* by formulating it into a hydrogel.

After extraction, the concentrated plant extract will be incorporated into a gel base prepared using Carboxymethyl Cellulose (CMC), a biocompatible and water-soluble polymer.

The combination of *Tridax procumbens* extract with CMC will result in a herbal hydrogel formulation, which is expected to enhance wound healing due to its natural and bioactive components (Cowan *et al.*, 1999).

The development and spread of multidrug resistant superbugs especially in the hospital environment, continues to be a burning global issue due to the indiscriminate and irrational use of antibiotics (Pitout *et al.*, 2008)

Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine. (Rabi *et al.*, 2008)

Medicinal plants possess antioxidant properties that protect the plant cells against the production of reactive oxygen species from cellular damage. Phenolic compounds present in the medicinal plants play a vital role in scavenging free radical species and protects human body against diseases (Kahkonen *et al.*, 1999)

The objective of the present work was to investigate the potential of *Tridax procumbens* Herbal Gel in the treatment of wound healing and for its antimicrobial activity. (Ashwini Nalawade *et al.*,)

Cellulose is the most inexhaustible natural polymer on earth and rich with hydroxyl groups which can easily form hydrogen bonding. It is characterized by its hydrophilicity, chirality, and biodegradability which is formed from repeated connection of D-glucose building blocks (Klemm, *et al.*, 2005).

Cellulose is suitable in the making of cellulose-based hydrogel and can be applied in various fields such as food additives, agriculture, civil engineering, cosmetic and pharmaceuticals. Cellulose is biorenewable material that represents one of the largest and renewable sources of environmentally friendly raw materials (Thakur & Thakur, *et al.*, 2014).

Carboxymethylcellulose (CMC) known as a polyelectrolyte cellulose derivative that contains carboxyl and hydroxyl groups (Benhalima, Ferfera-Harrar, & Lerari, *et al.*, 2017). This polymer chain is composed of anhydroglucopyranose units joined together by 1,4-glycosidic bonds. Moreover, CMC is regarded as anionic carboxymethyl ether which is prepared by reacting alkali cellulose with sodium monochloroacetate.

Hydrogels which are physically or chemically crosslinked with hydrophilic polymer networks are capable of absorbing large amount of water and retain significant amount of water in their structures. Hydrogels do not dissolve and can swell in the water. The rate of absorption of hydrogels in the water is influenced by the presence of functional groups such as alcohols, carboxyls, sulphonic acid on the polymer backbone (Dafader *et al.*, 2011).

In this study, calcium chloride was used as a crosslinker via ionic crosslinking. Many researchers investigated the potential of CMC in various applications especially in the making of CMC hydrogel that acts as superabsorbent. However, the making of superabsorbent from CMC using divalent salts as crosslinker has not been studied widely. It has been reported previously that cellulose hydrogel is capable in response to external stimuli such as solvents, temperature, pH, ionic force, electric field, light irradiation, and salt concentration (Sanna, *et al.*, 2013).

Methodology

Step1: Collection of plant material

Fresh *Tridax procumbens* plants samples were collected from local area such as Mahada colony and N2, Chh Sambhajinagar. Leaves were Hot air oven dried, powdered and extracted using methanol and using Rotary Evaporator apparatus and the antimicrobial Activity of the extract was analyzed.

Step2:- Preparation of Plant Extract

The collected fresh leaves of *Tridax procumbens* were freshly washed and then dried in Hot Air Oven. After drying the plant material, fine powder of it was made using mortar pestle. Dried plant material was stored in Air tight container. 10 gram of *Tridax procumbens* leaf powder was added in 50ml of methanol. After adding methanol in plant material, sample was filtered with help of filter paper and stored at rotatory shaker at 37 for 24 hours.

After incubation period, we have filter the sample with the help of filter paper, filtrate was collected in semi liquid form and transferred in eppendorf tubes. Check the antimicrobial activity against *E.coli* and *S.aureus*.

Step3:-Collection of microbes:- Bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* were used for the study and were collected from Shiv Chhatrapati College, Biotechnology Department.

Step4:-Preparation of Basal and soft Agar

Yeast extract : 0.3gm

NaCl : 0.5gm

Peptone : 0.5gm

pH : 7

Distilled water: 100ml

After preparation of Basal and soft Agar, autoclaved the media in 121°C for 15min. In Laminar Air flow, media was poured in petri plates under sterile condition. Let the media to solidify in petri plates. After the media was solidified, overnight culture of *E.coli*, *S.aureus* was added in media with the help of micro pipettes. Serial dilution of *Tridax procumbens* leaf sample was done in series of: 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} .

Step 5: Antibacterial activity:

Preparation of soft Agar tubes and basal agar plates. And small saline tubes. Or distilled water inoculate *S.aureus* and *E.Coli* culture with the help of loop in saline tubes. Adjust and check the O.D should be 0.1 or 0.01 or 0.8 or 0.9. Put 10ul from the 10^{-5} inoculated saline in soft Agar and then pour the soft Agar in basal agar plate, distribute uniformly by rotating it. Let the solidify air dry first. Then with the help of cork borer make wells (6mm). Add the *Tridax* extract (20ul). Store at 4 degree refrigerator for 30 min. Then in incubator for 37° C for 24 hours and observe the zone of inhibition next day. Measure the zone of inhibition.

To check the antimicrobial activity against *E.coli* and *S.aureus*, the wells were bored in petri plates of 1 cm depth with the help of well borer to load the samples. To these wells, the diluted samples of *Tridax procumbens* leaf extract were loaded, and labeled accordingly.

These plates kept in incubator at 37°C for 24 hour to check the activity of *Tridax procumbens* leaf extract against *E. coli* and *S. aureus*. The clear zone were checked around the bored holes where the sample were loaded, which indicates the inhibition of bacterial growth. *Tridax Procumbens* leaf extract was extracted by keeping the sample in rotary shaker for 24 hours at 37°C.

Step 6: Preparation of CMC (Formulation of Hydrogel)

In a beaker 2gm CMC powder, 10g citric acid was dissolved in 100 ml distilled water. Using magnetic stirrer the solution was stirred for 15 min to get homogenized solution. The leaf sample was added in solution and stirred using magnetic stirrer at room temperature for 30 min. The solution was poured in petri plates and incubated for 2 days at room temperature to get gel pads for wound healing. Remaining gel was used to prepare preservative coating for fruits. Preservative coating was formed by dipping the fruits in the gel. The coated fruits were checked for 8 days.

Step 6:-Preparation of Wound Healing Gel Pads:-

The prepared gel was poured in petri plates. 5ml, 10ml, 15ml, 20ml, 25ml of prepared gel was added to each petri plate respectively. These plates were incubated 48 hrs to obtain gel pads. As per the amount of gel poured, the gel pads with varied width were obtained.

Step 7:- Preparation of Preservative coating for fruits by Dipping method:-

The prepared gel was coated to the fruits. The layer was let to dry at room temperature. The preservative coating was formed on the fruits.

Step 8:- Phytochemical Tests-

Test for Terpenoids:- 0.2 g of each sample was mixed with 2 mL chloroform, 3 mL conc. H₂SO₄. Reddish-brown coloration indicated the presence of terpenoids

Test for flavonoids :- 4 mL of extract solution, 1.5 mL of 50% methanol solution a small magnesium chunk were warmed. 5-6 drops of concentrated HCl were added, red color was observed for flavonoids

Test for tannin / polyphenol:- To the diluted extract, 3-4 drops of 10% FeCl₃ were added, blue color was seen for gallic tannins and the presence of catechol tannin turned the solution green

Test for alkaloids: Meyer's test:- To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of pale yellow precipitate indicated the presence of alkaloids.

Test for saponins:- 2 g of powdered sample was boiled in 20 mL of distilled water. 10 mL of filtrate, 5 mL of distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins.

Result**Step 1- Collection of *Tridax Procumbens* leaves :**

Fig1. Collection of *Tridax Procumbens*



Fig .2 Washing of leaves



Fig. 3 Transfer into petri plate

Step 2: Preparation of *Tridax procumbens* leaves powder:**Fig 4.**Leaves after drying.**Fig5.** Powder of *Tridax Procumbeans*.**Step 3- Addition of powder in solvent :****Fig 6.** Addition of sample into methanol**Step 4 Filtration :****Fig7.** Filtrate the sample**Fig8.** Filtrate

Step 5: Extraction

Step 6: Collection of sample



Fig 9: Extract

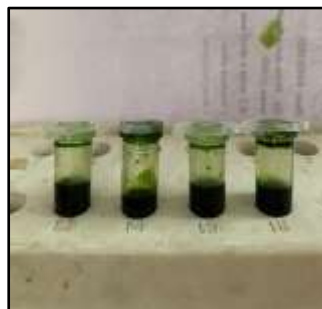


Fig10-Collection of sample in eppendroff tubes

Step 7-Antibacterial activity against *E.coli*

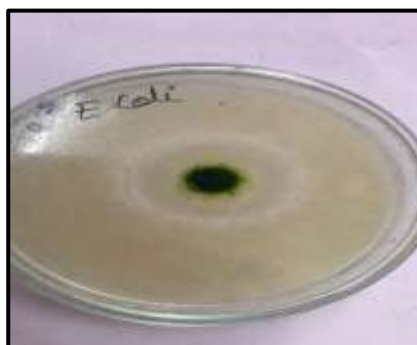


Fig. 11 . Antibiotic activity against *E. coli* : A clear zone of inhibition was observed, confirming antibacterial activity of the extract against *E. coli*.



As per Fig10: Antibacterial activity against *S.aureus* : A comparatively larger zone of inhibition was observed, indicating stronger antibacterial effect against *S. aureus*.

The percentage of Zone of inhibition was calculated using the formula:

$$\text{Zone of Inhibition} = \text{Total Diameter of Zone} - \text{Diameter of well or disk}$$

Step 8: Preparation of hydrogel:



Fig12:CMC and citric acid



Fig13: Addition of *T.procumbens*

Step 9 : Gel pad preparation.

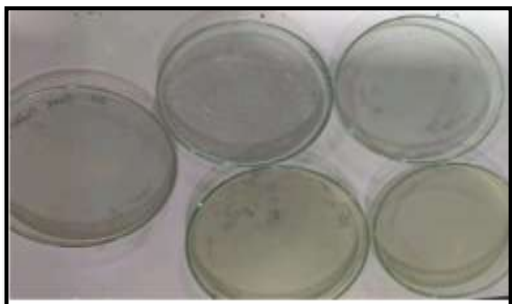


Fig14:-Gel added into petri plates



Fig15:-Gel pad formed

Step-10: Fruit coating :



Fig16:-Apple coated with CMC hydrogel



Fig17:-Apple without CMC hydrogel

Step11: Phytochemical screening:



Fig18:-Test Sample



Fig19:-Phytochemical Test

Sr.No	Phytochemical Test	Result
1	Alkaloids	Negative
2	Flavonoids	Positive
3	Tannins	Positive
4	Saponins	Positive
5	Terpenoids	Positive

Conclusion

- It was concluded that leaf extract of *Tridax procumbens* possesses the antimicrobial property against the bacterial samples checked for, *E. coli* and *S. aureus*.
- The clear zones were observed around the *Tridax procumbens* sample loaded in the culture media, which indicates the growth inhibition of the bacteria.
- The fruits coated with *Tridax procumbens* hydrogel preservative coating remained fresh and healthy for 8 days, whereas fruits without coating became stale.

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